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Genetics and biochemistry of 1,2-dichloroethane degradation

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Abstract

Dichloroethane (1,2-DCE) is a synthetic compound that is not known to be formed naturally. Nevertheless, several pure microbial cultures are able to use it as a sole carbon source for growth. Degradation of 1,2-DCE proceeds via 2-chloroethanol, chloroacetaldehyde and chloroacetate to glycolate. The genes encoding the enzymes responsible for the conversion of 1,2-DCE to glycolic acid have been isolated. The haloalkane dehalogenase and an aldehyde dehydrogenase are plasmid encoded. Two other enzymes, the alcohol dehydrogenase and the haloacid dehalogenase, are chromosomally encoded. Sequence analysis indicates that the haloacid dehalogenase belongs to the L-specific 2-chloroproprionic acid dehalogenases. From the three-dimensional structure and sequence similarities, the haloalkane dehalogenase appears to be a member of the α/β hydrolase fold hydrolytic enzymes, of which several are involved in the degradation of aromatic and aliphatic xenobiotic compounds.

Introduction

The bacterial degradation of chlorinated synthetic compounds is dependent on enzymes that recognize molecules that do not occur at significant concentrations in nature. The chlorinated compound with the largest production volume is 1,2-DCE. Its industrial synthesis started in 1923 and currently amounts up to more than 15 million tons annually worldwide. 1,2-DCE is mainly used for the synthesis of vinylchloride for PVC production, the synthesis of amines and other derivatives, and as a solvent in pharmaceutical synthesis. Environmental contamination usually is caused by accidents, such as leaking tanks or pipelines and accidental spillage. In addition, its normal use is often accompanied by diffuse emission via waste water and waste gases.

Despite its xenobiotic structure, several bacterial cultures that are able to utilize 1,2-DCE for growth have been isolated. Strains of at least three different genera of gram-negative bacteria are known to degrade DCE (Table 1). Stucki et al. (1983a) described the isolation of a *Pseudomonas* strain from a site close to a chemical plant. We have isolated and characterized 1,2-DCE degraders from several polluted sites in the Netherlands, including sediment from the river Rhine (Janssen et al. 1984; Van den Wijngaard et al. 1992). These facultatively methylotrophic organisms were classified as members of the genera *Xanthobacter* and *Ancylobacter*.

The catabolic pathway has been resolved with *Xanthobacter autotrophicus* GJ10 and strains of *Ancylobacter aquaticus* (Janssen et al. 1985; Van den Wijngaard et al. 1992), which led to the establishment of the route shown in Fig. 1. The first step is the hydrolytic conversion of 1,2-DCE to 2-chloroethanol. This intermediate is oxidized to chloroacetaldehyde by a periplasmic alcohol dehydrogenase. Further oxidation by an NAD-dependent aldehyde dehydrogenase yields chloroacetate, which is cleaved by a second hydrolytic dehalogenase to glycolate.

Both dehalogenases are produced constitutively, whereas the alcohol dehydrogenase and the aldehyde dehydrogenase are inducible (Janssen et al. 1985, 1987a; Van den Wijngaard et al. 1992). The haloalkane dehalogenase gene (*dhlA*) and a chloroacetalde-

Table 1. Organisms capable of growth on 1,2-dichloroethane or 2-chloroethanol.

Organism	Utilizes	Reference
Pseudomonas CE	2-chloroethanol	Stucki et al. 1983a
Pseudomonas DE1	1,2-dichloroethane, 2-chloroethanol, chloroacetic acid	Stucki et al. 1983b
Pseudomonas GJ1	2-chloroethanol	Janssen et al. 1984
Xanthobacter autotrophicus GJ10	1,2-dichloroethane, 2-chloroethanol, 1,3-dichloroethane, 1-chlorobutane	Janssen et al. 1984, 1985
Pseudomonas P4	1,2-dichloroethane	Scholtz et al. 1987
Pseudomonas US2	2-chloroethanol	Strotmann et al. 1990
Ancylobacter aquaticus AD20, AD25	1,2-dichloroethane, 2, chloroethanol, 2-chloroethylvinylether	Van den Wijngaard et al. 1992, 1993a

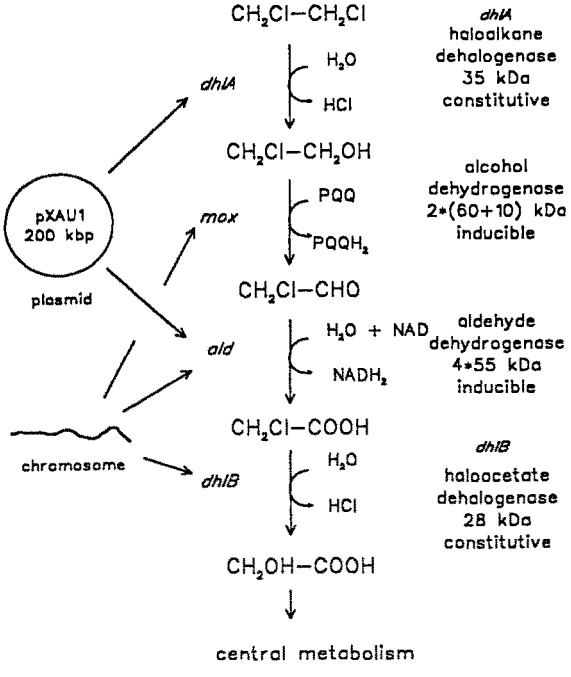

Fig. 1. The catabolic pathway of 1,2-dichloroethane in *X. autotrophicus* and *A. aquaticus*.

hyde dehydrogenase gene are plasmid encoded in *X. autotrophicus* GJ10 (Tardif et al. 1991; van der Ploeg et al. 1994). A similar plasmid is detected in *A. aquaticus* strains isolated on 1,2-DCE (Van der Ploeg JR, unpublished observation). The haloalkane dehalogenase, the modified aldehyde dehydrogenase, and the chloroacetate dehalogenase are only produced by strains of *Xanthobacter* that utilize 1,2-DCE (Janssen et al. 1987; Tardif et al. 1991).

Another route for 1,2-DCE degradation may be operative in a *Pseudomonas* strain that utilizes 1,2-DCE (Stucki et al. 1983a). No dehalogenase activity with 1,2-DCE was detected in this organism, and it was proposed that the first catabolic step is catalyzed by an oxygenase.

In this review, we focus on the genetics of 1,2-DCE degradation, and discuss the function of genes involved in the conversion to glycolate. Since adaptation to 1,2-DCE probably started (see below) with organisms capable of growing on chloroacetate or chloroethanol, we will first discuss chloroacetate conversion, followed by the other steps.

Chloroacetate dehalogenase

The capacity to use chloroacetate as a carbon source is widespread among gram-negative and gram-positive bacteria (Hardman & Slater 1981; Motosugi & Soda 1983). Such organisms are easily isolated from soil or sludge. Dehalogenation is the first catabolic step. It is catalyzed by hydrolytic enzymes producing glycolate. Several of these proteins have been isolated and different classes can be distinguished on basis of sequence comparison and stereoselectivity.

X. autotrophicus GJ10 produces a chloroacetate dehalogenase of 28 kDa. From the sequence (Van der Ploeg et al. 1991), the enzyme appears to be somewhat larger but similar to other L-specific 2-haloacid dehalogenases that convert L-2-chloropropionic acid to D-lactate, i.e. with inversion of configuration. Enzymes of this class have been identified in *Moraxella* and *Pseudomonas* strains (Schneider et al. 1991; Kawasaki et al. 1992; Jones et al. 1992; Murdiyatmo et al. 1992). The dehalogenase is constitutively produced in *Xanthobacter*, but higher levels are found in the stationary phase than in the exponential phase of growth

(Greer et al. 1989). Haloacid dehalogenase synthesis in *X. autotrophicus* GJ10 thus seems to be derepressed under carbon starvation. In agreement with this, mutant GJ10M41, which slowly utilizes ethanol as a result of a defective aldehyde dehydrogenase, showed much higher levels of the chloroacetate dehalogenase during growth on ethanol than the wild type strain (Janssen et al. 1987).

The enzymatic mechanism of 2-haloacid dehalogenation is unknown. It could be similar to that of haloalkane dehalogenase (Verschueren et al. 1993a; Pries et al. 1994), which is in agreement with inversion of configuration (see below). In haloalkane dehalogenase, tryptophan residues are involved in leaving group stabilization by binding the halide, as revealed by X-ray crystallography and fluorescence studies (Verschueren et al. 1993b). This is not the case in the *X. autotrophicus* GJ10 haloacid dehalogenase, since we found that tryptophan fluorescence is not quenched by halide ions or substrate (Kingma J & Janssen DB, unpublished results).

The haloacid dehalogenase seems to be responsible both for the utilization and detoxification of haloacetates. Mutants of *X. autotrophicus* GJ10 resistant to bromoacetate were found to produce increased levels of the enzyme, suggesting that resistance is caused by an increased rate of conversion (Van der Ploeg et al. 1991). In contrast, with *Pseudomonas putida* PP3, a chloropropionate utilizing organism, resistance to toxic levels of haloacetates was accompanied by a decreased level of haloacid dehalogenase, which was proposed to be caused by concomitant loss of an uptake dehalogenase, protein (Slater et al. 1985). Loss of haloacid dehalogenase activity was also found by Strotmann et al. (1990) in a mutant of the 2-chloroethanol utilizer *P. putida* US2 that is resistant to high concentrations of 2-chloroethanol.

Thomas et al. (1992) have suggested that environmental conditions could influence the deactivation of dehalogenase genes of *P. putida* PP3 by transposition of an element containing these genes to a cryptic state. The dehalogenase genes could in turn be activated again under different environmental conditions.

Aldehyde oxidation

A critical step in the degradation of halogenated aliphatics is the oxidation of the aldehydes that are produced as intermediates. The 2-haloacetaldehydes are bifunctionally reactive, and can form covalent products with amines and nucleophilic groups. This explains that 2-chloroethanol is very toxic for mutants of *X. autotrophicus* GJ10 that have lost aldehyde dehydrogenase activity (Janssen et al. 1987a; Tardif et al. 1991). Addition of 2-chloroethanol to *Xanthobacter* cultures isolated on methanol causes toxicity, even in a constructed strain that overexpresses the haloacid dehalogenase and grows on chloroacetate (Van der Ploeg et al. in preparation). Bacteria that degrade aldehydes or convert other compounds via aldehydes thus will have to maintain a low intracellular level of aldehydes.

Tardif et al. (1991) have shown that the NAD-dependent chloroacetaldehyde dehydrogenase of *X. autotrophicus* GJ10 is a plasmid encoded enzyme. We have cloned the gene and purified the enzyme from a recombinant strain which overexpresses it (Van der Ploeg et al., in preparation). The dehydrogenase is a tetrameric protein with 55 kDa subunits. Introduction of the aldehyde dehydrogenase gene in a mutant of *X. autotrophicus* GJ10 that has lost the whole catabolic plasmid (Janssen et al. 1987a) indeed led to transconjugants that are able to grow on 2-chloroethanol. Without the aldehyde dehydrogenase gene, chloroethanol was very toxic for the cured strain (Tardif et al. 1991).

The lack of a functional aldehyde dehydrogenase and the even higher reactivity of bromoacetaldehyde seem to be the cause of the fact that 1,2-DCE degraders do not utilize 1,2-dibromoethane for growth. 1,2-Dibromoethane is an excellent substrate for haloalkane dehalogenase and bromoacetate is rapidly hydrolyzed by haloacid dehalogenase. However, both 1,2-dibromoethane and 2-bromoethanol are toxic for strains of *X. autotrophicus* or *A. aquaticus* in the μM range. This can be used to select mutants that lack the alcohol dehydrogenase (Van den Wijngaard et al. 1993a) or the haloalkane dehalogenase (Van der Ploeg et al., submitted).

Alcohol dehydrogenase

Most bacterial cultures that grow on 1,2-DCE are facultative methylotrophs. One possible explanation for this may be the involvement of a periplasmic quinoprotein alcohol dehydrogenase in the oxidation of 2-chloroethanol. The enzyme is the same as the quinoprotein methanol dehydrogenases that function in methanol oxidation in gram-negative methylotrophs. Indeed, mutants lacking the enzyme do not grow on 2-chloroethanol or methanol (Van den Wijngaard et al. 1993a). The extracytoplasmic localization implies that

2-chloroethanol produced by the haloalkane dehalogenase is exported to the periplasm for further conversion. This is not uncommon in methylotrophic bacteria: methane, for example, is converted in the cytoplasm to methanol that is oxidized by the periplasmic methanol dehydrogenase. The other enzymes of 1,2-DCE metabolism seem to be located in the cytoplasm.

The alcohol dehydrogenase is a tetrameric enzyme (α_2 β_2) composed of 60 kDa and 10 kDa subunits. In *X. autotrophicus* GJ10, the synthesis of the enzyme is induced by methanol or 2-chloroethanol (Janssen et al. 1987). There is no indication for adaptational mutations being needed for 2-chloroethanol oxidation by the quinoprotein alcohol dehydrogenase. Chloroethanol is a very good substrate for the methanol dehydrogenase from *Methylobacterium* (Anthony & Zatman 1967).

Although the quinoprotein alcohol dehydrogenase plays a role in the conversion of 2-chloroethanol in *Xanthobacter* and *Ancylobacter* strains, it is clear that this specific class of alcohol dehydrogenases is not required for 2-chloroethanol oxidation. Not all 2-chloroethanol utilizing organisms are facultative methylotrophs. Non-methylotrophs growing on 2-chloroethanol may possess different various alcohol dehydrogenases, which may or may not be linked to NAD (Janssen et al. 1984; Strotmann et al. 1990). These alcohol dehydrogenases have not yet been well characterized.

Dehalogenation of 1,2-DCE

The first step in 1,2-DCE metabolism is catalyzed by haloalkane dehalogenase, a soluble 35 kDa protein of 310 amino acids that hydrolytically cleaves carbon-chlorine bonds to produce alcohols and halides. The dehalogenase is identical in different isolates of *X. autotrophicus* and *A. aquaticus* (Van den Wijngaard et al. 1992). Certain strains of *A. aquaticus* are able to grow on (and were selected on) 2-chloroethylvinylether; these strains also produce a dehalogenase with the same amino acid sequence, but at a much higher level (Van den Wijngaard et al. 1992, 1993a).

The regulation of haloalkane dehalogenase synthesis is simple: the enzyme is not induced but expressed constitutively at high levels during each growth phase and on all media tested. There are two consensus *E. coli* promoter sequences preceding the *dhlA* gene in *X. autotrophicus* GJ10 (Janssen et al. 1989). This can be regarded as a sign of evolutionary primitivity: a regulation system would require, in addition to the dehalogenase, a second protein (the sensor protein) that can recognize 1,2-DCE. This apparently has not yet evolved. It is noteworthy that dichloromethane dehalogenase (La Roche et al. 1991) and haloalkane dehalogenases converting long-chain chloroalkanes (Scholtz et al. 1987; Janssen et al. 1987b) are induced by their substrates.

The substrate range of haloalkane dehalogenase is very broad and includes a number of environmentally important compounds (Table 2). The best substrate found so far is the nematocide 1,2-dibromoethane, a compound that has widely been used as a soil fumigant for citrus crops and that is a frequent groundwater contaminant and has a specificity constant of k_{cat}/K_m of 480 $\mu M^{-1}S^{-1}$. The V_{max} values with 1,2-DCE and 1,2-dibromoethane are both about 6 U/mg protein, but the K_m for 1,2-dibromoethane is 7 μM, which is 100-fold lower than with 1,2-DCE.

The mechanism of haloalkane dehalogenase was studied by X-ray crystallography and isotope incorporation studies, and involves nucleophilic attack by the side chain carboxylate of Asp124. This is followed by hydrolysis of the resulting covalent alkyl-enzyme intermediate by attack of water at the carbonyl carbon of the esterified Asp124 (Verschueren et al. 1993a, b; Pries et al. 1994a). There is a distinct halide binding site, which was identified by X-ray crystallography and fluorescence measurements (Verschueren et al. 1993b). It is formed by two tryptophan residues, of which the hydrogen atoms connected to the ring nitrogens are responsible for halide binding. The site has a much higher affinity for bromide than for chloride, which can explain the lower K_m value of 1,2-dibromoethane as compared to 1,2-DCE. The active site is a cavity located between the two domains of haloalkane dehalogenase. One of the tryptophan residues involved in halide binding (Trp125) is located in the main domain, the other (Trp175) in the cap that lies on top of the main domain. Thus, both domains contribute to the specificity of catalysis of carbon-halogen bond cleavage. The enzyme clearly is not a general hydrolytic protein that fortuitously also converts chlorinated substrates.

The evolutionary origin of the haloalkane dehalogenase is unknown, but the main domain is similar to a number of hydrolytic enzymes that are classified as α/β hydrolase fold enzymes (Ollis et al. 1992). Other enzymes of this class can be identified by sequence analysis, and several appear to be involved

Table 2. Substrate range of haloalkane dehalogenase.

Compound	% Activity[a]	Use, relevance
1,2-dichloroethane	100	vinylchloride synthesis, solvent
chloromethane	28	blowing agent, naturally produced
dichloromethane	9	solvent
ethylchloride	24	cooling agent
1-chloropropane	48	–
1,3-dichloropropane	87	–
1,2-dichloropropane	0.6	chemical waste
1,3-dichloropropene	96	nematocide
2-chloroethylvinylether	12	waste product
2-chloroethylmethylether	6	waste product
epichlorohydrin	8	pharmaceutical synthesis, resins
2-chloroethanol	< 1	solvent
1,2-dibromoethane	94	nematocide, gasoline
methylbromide	13	nematocide
dibromomethane	94	naturally produced
ethylbromide	24	–
2-bromoethanol	24	–

[a] Activities are given at a substrate concentration of 5 mM. Values are expressed as the percentage of activity found with 1,2-dichloroethane, which was 6 μmol/min/mg of protein (turnover number k_{cat} 3.9 substrate molecules/enzyme molecule per second). Data were obtained as described by Keuning et al. (1985).

in the bacterial degradation of xenobiotic compounds. The enzymes catalyze hydrolytic reactions by covalent catalysis (Fig. 3, see below). The nucleophilic amino acid can be a cysteine, a serine, or an aspartic acid. These residues are at a conserved topological position in the structure. An alignment of a part of the sequence around the nucleophilic residue of the hydrolases is shown in Fig. 2. From this, a consensus hydrolase box sequence Val-Gly-X-Nu-X-Gly-Gly can be derived.

Recently, we have studied the adaptation of the dehalogenase to novel substrates by selecting mutants that grow on 1-chlorohexane, a compound hardly hydrolyzed by the wild type enzyme (Pries et al., 1994b). The results of selection experiments indicated that generation of short direct repeats in the N-terminal part of the cap domain plays an important role in the adaptation of the dehalogenase to new substrates. Similar short repeats are present in the cap domain of the wild type enzyme, which seems to reflect recent evolutionary events (Pries et al., 1994b,c).

This suggests that haloalkane dehalogenase may have evolved from an enzyme that converts related compounds of natural origin. It is well conceivable that the enzymes that play a role in the degradation of synthetic chloroaliphatics such as 1,2-DCE are related to enzymes involved in the degradation of naturally produced halogenated compounds, of which several are low molecular weight aliphatics (Gschwend et al. 1985). Little is known about the biodegradation of these compounds, however.

Evolution of a 1,2-DCE degradative pathway

The 1,2-DCE degrading organisms that have been isolated may have evolved from facultative methylotrophs that are capable of utilizing haloacetates by acquisition of a plasmid encoding the haloalkane dehalogenase and the aldehyde dehydrogenase. How then did this plasmid evolve? Possibly, chromosomally encoded genes were transferred to plasmids during strong selection pressure for the function of these genes. This is in agreement with the observation that many properties that have recently come under selection pressure are encoded on plasmids, such as utilization of xenobiotics, heavy metal resistance and resistance to antibiotics. How this happens is not known (Van der Meer

A

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                    ↓ ↑
P22643    DhlA  RNITLVVQDWGGFLGLTLPMAD  137
D14594    LinB  DRVVLVVHDWGSALGFDWARRH  121
D90422    DehH1 ERFHLVGHDRGGRTGHRMALDH  118
P19076    DmpD  EQADLVGNSFGGGIALALAIRH  120
P17548    BphD  EKAHLVGNSMGGAGALNFALEY  125
P23106    XylF  QQGDIVGNSFGGGLALALAIRH  119
P23133    TodF  DRVDLVGNSFGGALSLAFAIRF  118
P27100    TcbE  GGVAVIGYCLGGALAYEVAAEG  136
P27136    TfdE  GRVAVVGYCLGGALAFDVAARS  136
P11453    ClcD  GKVGLVGYCLGGALAFLVAAKG  136
                . .**   **.... * .
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B

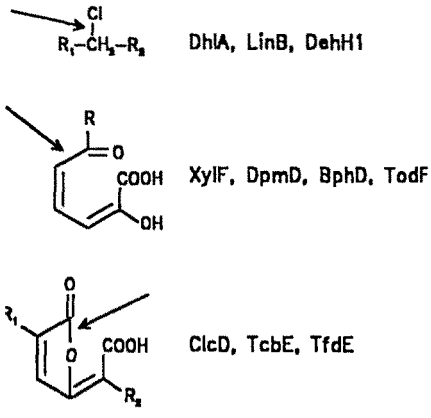

Fig. 2. Sequence comparison around the nucleophilic residue of α/β-fold hydrolases involved in biodegradation (A) and substrates converted (B). The abbreviations are: DhlA, haloalkane dehalogenase from *X. autotrophicus* GJ10 (Janssen et al. 1989); LinB, γ-hexachlorocyclohexane dehalogenase from *Pseudomonas paucimobilis* (Nagata et al. 1993); DehH1, haloacetate dehalogenase from *Moraxella* (Kawasaki et al. 1992); DmpD, 2-hydroxymuconic semialdehyde hydrolase from *Pseudomonas* (Nordlund & Shingler 1990); BphD, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase from *Pseudomonas* (Kimbara et al. 1989); XylF, 2-hydroxymuconic semialdehyde hydrolase from *Pseudomonas putida* (Horn et al. 1991); TodF, 2-hydroxy-6-oxohepta-2,4-dienoate hydrolase from *Pseudomonas putida* (Menn et al. 1991); TcbE, dienelactone hydrolase from *Pseudomonas* P51 (van der Meer et al. 1991); TfdE, dienelactone hydrolase from *Alcaligenes eutrophicus* (Perkins et al. 1992); ClcD, dienelactone hydrolase from *Pseudomonas* B13 (Frantz et al. 1987). Symbols: *, identity for 8 or 9 sequences. •, identity for 5, 6 or 7 sequences. ↓, nucleophilic residues; ↑, tryptophan residue involved in substrate and halide binding in haloalkane dehalogenase (Veschueren et al. 1993b). From the alignment, the derived consensus amino acid sequence for the hydrolase box is: Val-Gly-X-Nu-Gly-Gly, where Nu is the nucleophilic residue involved in catalysis.

et al. 1992). Several catabolic genes or operons located on plasmids are flanked by insertion sequences and thus are part of a catabolic transposon (for examples, see the review of Wyndham et al. elsewhere in this volume). These DNA segments may have been transferred from the chromosome to a plasmid.

There is no direct evidence for such a process for the plasmid encoded genes involved in 1,2-DCE degradation, however. Interestingly, there is an open reading frame downstream of the haloalkane dehalogenase gene that encodes a protein with strong sequence similarity to *rhs* element encoded H-rpt proteins of *E. coli*, which are involved in recombination processes (Stroeher et al. 1992; Zhao et al. 1993). Thus, it is possible that this 3' open reading frame was involved in movement of the *dhlA* gene to the *Xanthobacter* plasmid from some other replicon.

Horizontal transmission of the pXAU plasmid to other facultative methylotrophs (*Xanthobacter* or *Ancylobacter*) that produce a haloacetate dehalogenase may have led to a variety of 1,2-DCE and 2-chloroethylvinylether degrading organisms, as can now be isolated from environmental samples. Experimentally, this transmission has not yet been demonstrated, however.

The origin of the plasmid-encoded haloalkane dehalogenase and chloroacetaldehyde dehydrogenase genes in unknown. It is likely that these enzymes are specifically adapted to xenobiotic substrates, i.e. underwent mutations that were maintained and selected for as a result of the presence of synthetic chlorinated compounds in environmental samples. It thus remains to be determined from which proteins the present specialized enzymes have evolved and which mutations took place during their adaptation.

Prospects for genetic improvement

The persistence of a number of halogenated aliphatic compounds in the environment and the apparent recalcitrance of these compounds in biological treatment systems is largely related to the low microbial potential to use these compounds for growth. The substrate range of dehalogenases is one of the factors that influence the range of compounds that can be degraded. Furthermore, the kinetics of initial catabolic enzymes influence the growth kinetics of organisms.

Chemostat studies with 1,2-DCE degraders (Van den Wijngaard et al. 1993b) showed that the kinetic properties of haloalkane dehalogenase have consequences for the K_s of the organism. A low k_{cat} (turnover number), a low enzyme concentration, and a high K_m of the dehalogenase lead to a high Monod constant (K_s) for 1,2-DCE utilization (Van den Wijngaard et al. 1993b). At low substrate concentration ($S << K_m$), the rate of substrate removal by an enzyme is given by k_{cat}/K_m (selectivity) and a higher value will lead to lower steady state substrate concentrations and improved removal in bioreactors. Both in natural environments and in treatment systems, the production of increased levels of dehalogenase or an enzyme with higher affinity would yield reduced concentrations of toxicants.

The haloalkane dehalogenase is certainly not a perfect enzyme. The k_{cat} with 1,2-DCE is only 6 s^{-1}, and the k_{cat}/K_m is 5100 $s^{-1}M^{-1}$. This is significantly higher than the K_s value of the organism, which indicates that the rate of 1,2-DCE conversion obtained at a substrate concentration equal to the K_m of the enzyme is higher than the rate needed for growth at 0.5 μ_{max}.

It is highly conceivable that the dehalogenase can evolve further to improve its kinetics with 1,2-DCE or other substrates. This is also suggested by the finding that the same enzyme is present in organisms isolated on 1,2-DCE and 2-chloroethylvinylether: it seems unlikely that the same amino acid sequence yields a protein with optimal activity for two such different substrates. Furthermore, the current enzyme seems to be more suitable for brominated compounds than for chlorinated substrates. It is possible that mutants with improved kinetic constants can be constructed by site-directed mutagenesis. The availability of detailed structural information (Verschueren et al. 1993a) and site-directed mutagenesis and expression vectors (Schanstra et al. 1993) will help us to explore these possibilities.

Another factor influencing applicability of dehalogenase producing organisms is the substrate range. In fact, there is some correlation between the substrate range of the dehalogenase and the recalcitrance of various structurally similar chloroalkanes. Thus, compounds such as 1,1-DCE,1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,2-dichloropropane, and 1,2,3-trichloropropane are hardly converted by the dehalogenase and have never been found to be utilized as growth substrates by bacteria. The engineering or selection of dehalogenases with expanded catalytic utility thus would be an important step towards the construction of organisms that grow on chlorinated aliphatics that are at present recalcitrant. The availability of suitable process technologies (Morgan & Watkinson 1989; Stucki et al. 1992) for the removal of chlorinated compounds from waste streams or contaminated water using specific strains indicates that the acquisition of improved biocatalysts is a key step towards enlarging the application area of biological remediation technologies.

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